Check for updates

RESEARCH ARTICLE

Alexiteric activity of *Costus pulverulentus* C. Presl., *Desmodium adscendens* (Sw.) DC., *Begonia glabra* Aubl. and *Equisetum bogotense* on the poison of *Bothrops asper* (equis)

[version 1; peer review: 2 not approved]

Wilson Tapia[1], Katy Garzón[2], Nelly Granda[1], Bence Mátyás[3]

[1]Environmental Research, Bioprospecting Secondary Metabolites and Animal Biotechnology, Universidad Politécnica Salesiana, Quito, Ecuador
[2]Herpetological Foundation "Gustavo Orcés, Vivarium de Quito, Quito, Ecuador
[3]Grupo de Investigación Mentoria y Gestión del Cambio, Universidad Politécnica Salesiana, Cuenca, Ecuador

v1 **First published:** 01 Feb 2018, **7**:136
https://doi.org/10.12688/f1000research.13528.1
Latest published: 01 Feb 2018, **7**:136
https://doi.org/10.12688/f1000research.13528.1

Abstract

Background: According to the Ecuadorian ancestral knowledge, antivenom treatment can be carried out using medicinal plants; there are *in vitro* studies in which alexiteric activity has been proven in many plant species through the assay of reduction of indirect hemolytic activity in blood agar plate-phosphatidylcholine. The cases of snake bites "equis" on the Ecuadorian coast represent the largest number of reported ophidian accidents, which usually occur in remote areas of health centers or hospitals, making patient care not timely. The species *Costus pulverulentus* C. Presl, *Desmodium adscendens* (Sw.) DC., *Begonia glabra* Aubl., and *Equisetum bogotense* are considered plants with antivenom potential (alexiteric) and have been used by various Ecuadorian ethnicities.
Methods: In the present investigation, the indirect anti-hemolytic activity *in vitro* of dry extracts of these four-plant species was evaluated, as an indicator of alexiteric activity, on three "pools" of *Bothrops asper* (equis) snake venom provided by the Vivarium of Quito (Quito, Ecuador).
Results: Alcoholic extracts of the four-species showed neutralizing activity of the venom, with some differences according to the treatment and origin (composition) of the venom. The aqueous extract of *B. glabra* presented antihemolytic activity, unlike the other species.
Conclusions: We conclude that the alcoholic extracts of the four-species presented activity with some differences according to treatment and origin (composition) of the poison, and the aqueous extract of *B. glabra* presented anti-hemolytic activity unlike the other species whose activity was null.

Open Peer Review

Approval Status ✗ ✗

	1	2
version 1 01 Feb 2018	✗ view	✗ view

1. **Juan J. Calvete**, Superior Council of Scientific Investigations (CSIC), Valencia, Spain

2. **Andre Lopes Fuly**, Federal Fluminense University, Niteroi, Brazil

Any reports and responses or comments on the article can be found at the end of the article.

Keywords
Bothrops asper alexiteric activity; Desmodium adscendens, Begonia glabra; Costus pulverulentus.

Corresponding author: Bence Mátyás (matyasbence@agr.unideb.hu)

Author roles: Tapia W: Investigation, Methodology, Writing – Original Draft Preparation; **Garzón K**: Conceptualization, Writing – Original Draft Preparation; **Granda N**: Investigation, Writing – Original Draft Preparation; **Mátyás B**: Project Administration, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

Copyright: © 2018 Tapia W *et al*. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

How to cite this article: Tapia W, Garzón K, Granda N and Mátyás B. **Alexiteric activity of *Costus pulverulentus* C. Presl., *Desmodium adscendens* (Sw.) DC., *Begonia glabra* Aubl. and *Equisetum bogotense* on the poison of *Bothrops asper* (equis) [version 1; peer review: 2 not approved]** F1000Research 2018, **7**:136 https://doi.org/10.12688/f1000research.13528.1

First published: 01 Feb 2018, **7**:136 https://doi.org/10.12688/f1000research.13528.1

Introduction

Ophidic accident is defined as injury resulting from the bite of a poisonous snake that causes toxic effects on the victim. Snake venom is a complex mixture of protein-enzymatic substances and non-protein substances that produce pain, edema, and drop in blood pressure, hemolysis, proteolysis and alterations in blood coagulation[1]. It can be considered a public health problem both by the effects they generate and by the complications derived from inopportune treatment, causing disability or even death of the patient.

In Ecuador, 1953 cases were reported during the year 2016 and 313 cases have been reported between January and March 2017[2]. In the littoral region, the most common species is *Bothrops asper* (equis), while *Bothrops atrox* (pitalala) is the most common in the Amazon region[3].

In traditional medicine, many plants have been used to combat the effects of venomous snake bites; they are called alexiteric because they are used to mitigate the effects of the venom of poisonous animals[4]. Indirect anti hemolytic activity of *Equisetum bogotense* Kunth has shown a reduction in the hemolytic effect induced by *B. asper* venom[5]. The species *Costus pulverulentus* C. Presl, *Desmodium adscendens* (Sw.) DC., *Begonia glabra* Aubl. and *Equisetum bogotense*, listed in the book "Useful Plants of Ecuador"[6], are considered plants with antiophidic potential.

The present study evaluated the anti-hemolytic effect *in vitro*, used as an indicator of alexiteric activity, of these plant species to contribute in the search for solutions to the problem triggered by ophidian accidents.

Methods

Obtaining plant samples

The plant samples were collected in areas of northwestern Pichincha (Table 1) and species were identified at the National Herbarium of Quito (Quito, Ecuador).

The parts used for the plant species, according to the traditional use of the inhabitants of the area, to be tested are as follows:

- *Costus pulverulentus* C. Presl.: rhizomes
- *Desmodium adscendens* (Sw.) DC.: whole plant
- *Begonia glabra* Aubl.: leaves
- *Equisetum bogotense* Kunth.: whole plant

Preparation of extracts

Plant samples were dried in an oven at 37 degree C for 48 h, and then were ground to a particle size of 5 mm from which extracts were prepared by maceration with solvents of increasing polarity (n-hexane < alcohol < water). Hexane extract: 25 g of dried plant material was macerated with 350 mL of n-hexane at room temperature for 48 h. Then it was filtered and concentrated to dryness in rotary evaporator at 40 degree C and stored in an amber vial. The dry residue of this extraction was used to obtain the following alcoholic extract. Alcoholic extract: The dry residue from the previous extraction was macerated with 350 mL of absolute ethyl alcohol at room temperature for 48 h. Then it was filtered and concentrated to dryness in rotary evaporator at 40 degree C and stored in an amber vial. The dry residue of this extraction was used to obtain the following aqueous extract. Aqueous extract: The dry residue from the previous extraction was macerated with 350 mL of distilled water at room temperature for 48 h. Then it was filtered and concentrated to dryness in a water bath at 40 degree C and stored in an amber vial.

Obtaining the poison of *B. asper*

The sample of poison was obtained by manual milking of specimens in captivity (Figure 1), carried out by the staff of the Herpetological Foundation "Gustavo Orcés" (Vivarium of Quito, Quito, Ecuador). These specimens corresponded to three "pools" of *B. asper* venom from of the Ecuadorian coast, according to the specifications in Table 2.

Table 1. Sampling sites with their Universal Transverse Mercator (UTM) coordinates.

Species	Geographical coordinates (UTM)		Sector
Costuspul verulentusC. Presl.	17 N 719392 m E	8862 m N	Km 113 Vía Calacalí Puerto Quito
	17 N 719393 m E	8861 m N	
	17 N 719395 m E	8855 m N	
Begonia glabra Aubl.	17 S 747627 m E	9979137 m S	Mindo
	17 S 747625 m E	9979139 m S	
	17 S 747623 m E	9979139 m S	
Desmodium Adscendens (Sw.) dc.	17 N 719391 m E	8858 m N	Km 113 Vía Calacalí Puerto Quito
	17N 719393 m E	8855 m N	
	17N 719393 m E	8855 m N	
Equisetum bogotense Kunt.	17N784560 m E	9982670 m S	Quito Mercado de Nayón

Figure 1. Manual milking and samples of *Bothrops asper* specimens.

Table 2. Origin of *Bothrops asper*.

Location	*Pool of venom*
Mindo Pedro V. Maldonado Puerto Quito Sto. Domingo de los Tsáchilas	V1
Esmeraldas Manabí	V2
Bolívar	V3

The pools of venom were stored in a vacuum desiccator protected from light and refrigerated at 4°C until the tests were carried out in the laboratories of the Life Sciences Area of the Universidad Politécnica Salesina, Campus Giron- Quito.

Determination of the Minimum Hemolytic Dose (MHD)

The technique described in 7 was followed. Briefly, on a Petri dish containing blood-phosphatidylcholine agar that was prepared by mixing 1% agar, 0.1 mm CaCl2, 0.33% egg yolk and 1.2% washed erythrocytes[8], 15 μL of each dilution of venom, in physiological saline (4.76%, 2.44%, 1.23%, 0.62% v/v), was sown. The petri dishes were incubated for 20 h at 37°C. The halo of hemolysis was measured in mm. The lowest dose of venom that generated a 10 mm halo was taken as MHD.

Determination of the indirect antihemolytic activity of the extracts

The technique described in 8 was followed. Briefly, plant extract (1.6μg/mL) was pre-incubated with the MHD of the ophidian venom (ratios of 1: 7; 1:10; 1:20; poison: extract) for 30 minutes at 37°C. μL. 15 μL of this mixture was plated on a 6 mm-diameter petri plate containing blood-agar-phosphatidylcholine and incubated for 20 h at 37°C. The test was carried out in triplicate for each extract and for each concentration.

Statistical analysis

Analysis of variance was performed and after verifying significant differences, the Tukey test was applied using the Infostat/L program, version 11.1.2014, with a significance of 95%.

Results

Minimum hemolytic dose (MHD)

Table 3 shows the values obtained for the minimum dose hemolytic (MIDH) poison of B. asper from different areas of the Ecuadorian coastal region. It is shown that pool V2, from Esmeraldas an Manabí province, presents greater hemolytic activity compared with V1 and V3, from the Northwest of the Pichincha and Bolivar provinces, respectively. These two pools have the same activity; however, must consider that the three poisons are mixtures obtained from specimen captive males and females, adults and young from each of the above locations.

Example photographs of the MHD can be seen in Supplementary File 1.

Indirect antihemolytic activity

Table 4 shows the results, assigning the sign (+), for the treatment that reduced the diameter of hemolysis produced by the MHD of each pool by 100%. The alcoholic extract of *E. bogotense* has very good inhibitory activity of the hemolytic effect produced by the venom of *B. asper*, which has also been shown in previous research on *Bothrops atrox* (Yarlequé amp; *et al.*, 2012). However, in the case of *B. glabra*, its aqueous extract as well as its alcoholic extract is also effective. This shows that this plant is the most effective species of all tested.

The alcoholic extracts of *E. bogotense* and *D. adscendens* present antihemolytic activity in all treatments and for the three pools of venom, which shows they are the most active species against the hemolytic effect of *Bothrops asper* venom. Neither the aqueous nor the hexane extract of these two species demonstrate this activity.

Dataset 1. The minimum hemolytic dose raw data for the four plant species against the three pools of venom

http://dx.doi.org/10.5256/f1000research.13528.d191501

The antihemolytic activity percentages for all extracts of plant species, and concentrations of the three venom pools.

Dataset 2. Raw data for the MHD for the three pools of venom

http://dx.doi.org/10.5256/f1000research.13528.d191502

Table 3. Minimum hemolytic dose of *Bothrops asper* venom from Ecuadorian littoral region.

Source of *Bothrops asper*	Pool venom code	MIDH d(μg/μL)
Mindo, Pedro V. Maldonado, Puerto Quito, Santo Domingo de los Tsáchilas	V1	22.9
Esmeraldas Manabí	V2	6.1
Bolívar	V3	22.9

Table 4. Reduction in the hemolytic effect of *Bothrops asper* venom with various plant species. ((++) is 100% inhibition; (+) is lower activity; (-) is zero activity).

Species	Treatment (Vx:E)	Poison Pool	Extract		
			aqueous	alcoholic	hexanic
B. glabra	1:7	V1	++	++	-
		V2	++	+	-
		V3	+	+	-
	1:10	V1	++	++	-
		V2	++	+	-
		V3	+	+	-
	1:20	V1	++	++	+
		V2	++	+	+
		V3	+	+	-
C. pulverulentus	1:7	V1	-	++	-
		V2	-	-	-
		V3	-	+	-
	1:10	V1	-	+	-
		V2	+	++	-
		V3	-	-	-
	1:20	V1	+	++	-
		V2	-	+	-
		V3	+	+	-
D. adscendens	1:7	V1	-	++	-
		V2	-	++	-
		V3	+	++	-
	1:10	V1	-	++	-
		V2	-	++	-
		V3	+	++	-
	1:20	V1	-	++	+
		V2	-	++	+
		V3	+	++	-
E. bogotense	1:7	V1	-	++	-
		V2	-	++	-
		V3	-	++	-
	1:10	V1	-	++	-
		V2	-	++	-
		V3	-	++	-
	1:20	V1	-	++	-
		V2	-	++	-
		V3	-	++	-

Conclusions

The aqueous and alcoholic extracts of *B. glabra* show antihemolytic activity for all the treatments. In particular, the aqueous extract is effective against the anti-hemolytic effect of V_1 and V_2 and the alcoholic only against V_2. However, no extract of *B. glabra* demonstrated efficacy against the V3 pool. The species with the least alexiteric activity is *C. pulverulentus*, although it is active in front of the pool V1.

Although there are differences in activity according to extracts, plant species and origin of venom, the four evaluated plant species could be used in the prevention of the symptoms of a bothrophic accident. Taking into account their ancestral use, namely their consumption, this may be a preventive measure against the effects of *Bothrops asper* venom in the absence of adequate and timely professional medical treatment.

Ethical statement

All efforts were made to ameliorate any suffering to the animals following the protocol described by the World Health Organization[9] in accordance with the Ecuadorian code of practice for the care and use of animals for scientific purposes of the Ministry of Environment of Ecuador. Our experiments were approved by the Ministry of Environment of Ecuador (research permit number: No. 07-2014-IC-FAU-FLO-DPAP-MA).

Data availability

Dataset 1. The minimum hemolytic dose raw data for the four plant species against the three pools of venom. The antihemolytic activity percentages for all extracts of plant species, and concentrations of the three venom pools. DOI, 10.5256/f1000research.13528.d191501[10]

Dataset 2: Raw data for the MHD for the three pools of venom. DOI, 10.5256/f1000research.13528.d191502[11]

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

Supplementary material

Supplementary File 1: Examples of photographs of the results. MIDH in V1, MIDH in V2, MIDH in V3, Treatment V1-extract aqueous E. bogotense Kunth., Treatment V2-extracto aqueous D. adscendens (Sw.) DC., Treatment V3-extract aqueous C. pulverulentus C. Presl, Treatment V1-extracto alcoholic E. bogotense Kunth., Treatment V2-extracto alcoholic D. adscendens (Sw.) DC., Treatment V3-extract alcoholic de C. pulverulentus C. Presl, Treatment V1-extract hexane B. glabra Aubl., Treatment V2-extract hexane de B. glabra Aubl., Treatment V3-extract hexane E. bogotense Kunth.

Click here to access the data.

References

1. Pineda D: **Accidentes por animales venenosos.** Bogotá: Instituto Nacional de Salud. 2006.
2. Cueva, Zamora X: **Mordedura de serpientes.** *SMordedura de serpientes.* Quito: Universidad Central del Ecuador. 2010.
3. Ministerio de Salud Pública del Ecuador: **Manual de normas y procedimientos sobre prevención y tratamiento de accidentes ocasionados por mordedura de serpientes.** 2008.
   Reference Source
4. López J: **Plantas alexitéricas. Antídotos naturales contra la picadura de serpientes venenosas.** *Medicina naturista.* 2009; 17–24.
   Reference Source
5. Barranco W: **Evaluación biológica preliminar de extractos vegetales utilizados en la medicina natural en ls Sierra Nevada de Santa Marta contra el veneno de Bothrops asper.** Duazary. 2012; 140–150.
   Reference Source
6. Ríos M: **En Plantas útiles del Ecuador.** Quito: Abya-yala. 2007.
   Reference Source
7. Camargo FJ, Torres AM, Tressens SG, *et al.*: **Inhibición de la actividad hemolítica del veneno de *Bothrops neuwiedi diporus* Cope (yarará chica) por extractos de plantas del nordeste argentino.** Corrientes: Universidad Nacional del Nordeste. Obtenido de Comunicaciones científicas y tecnológicas. 2005.
   Reference Source
8. Camargo F, Dellacassa E, Ricciardi A, *et al.*: **SDS-PAGE una herramienta útil en la evaluación preliminar de actividad alexítera de extractos vegetales.** Boletin latinoamericano y del caribe d eplantas medicinales y aromáticas. 2011; **10**(5): 429–434.
   Reference Source
9. World Health Organization: **WHO Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins.** World Health Organization, 20 Avenue Appia, 1211 Geneva 27. 2016.
   Reference Source
10. Tapia W, Garzón K, Granda N, *et al.*: **Dataset 1 in: Alexiteric activity of Costus pulverulentus C. Presl., Desmodium adscendens (Sw.) DC., Begonia glabra Aubl. and Equisetum bogotense on the poison of Bothrops asper (equis).** *F1000Research.* 2018.
    Data Source
11. Tapia W, Garzón K, Granda N, *et al.*: **Dataset 2 in: Alexiteric activity of Costus pulverulentus C. Presl., Desmodium adscendens (Sw.) DC., Begonia glabra Aubl. and Equisetum bogotense on the poison of Bothrops asper (equis).** *F1000Research.* 2018.
    Data Source

Open Peer Review

Current Peer Review Status: ✗ ✗

Version 1

Reviewer Report 16 May 2018

https://doi.org/10.5256/f1000research.14690.r33719

© **2018 Fuly A.** This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

✗ **Andre Lopes Fuly**
Department of Molecular and Cellular Biology, Federal Fluminense University, Niteroi, Brazil

The manuscript reports inhibitory effects of plants against hemolysis induced by the venom of *Bothrops asper* snakes, and it postulates the use of plants as antivenom. However, the results are too preliminary to mention that such plants could be used to treat snake bites, since envenomation by Bothrops species produces a myriad of toxic effects such as tissue necrosis, edema and hemorrhage. At the least, more activities should be tested instead of only one; the authors choose an *in vitro* activity, the indirect hemolytic test, which is not the best to perform in order to postulate a molecule as antivenom. Some *in vivo* tests must be performed as well to strengthen the manuscript.

Thus, I must reject manuscript as it is presented.

Is the work clearly and accurately presented and does it cite the current literature?
No

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: toxinology, natural products, coagulation, platelet aggregation

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 07 March 2018

https://doi.org/10.5256/f1000research.14690.r31166

© 2018 Calvete J. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Juan J. Calvete
Institute of Biomedicine of Valencia (IBV), Superior Council of Scientific Investigations (CSIC), Valencia, Spain

Authors should provide an overview of the major life-threatening effect of an Ecuadorian bothropic snakebite. Major components of Bothrops snakes, particularly *B. asper* and *B. atrox* are myonecrotic and immflamatory phospholipase A_2s, hemorrhagic metalloproteases, and thrombin-like enzymes, whose substrates include components of the blood clotting system such as factors XII, X, and fibrinogen. What is the relevance of venom-induced hemolysis? This should be clarified, and, when available, the hemolytic toxins briefly described.

Table 4: The results of the reduction in the hemolytic effect of different mixtures of various plant species extracts pre-incubated with the MHD of the *B. asper* venom (ratios of 1: 7; 1:10; 1:20; poison: extract) should be better quantified (-, +, and ++ is too vague..).

"The cases of snake bites "equis" on the Ecuadorian coast represent the largest number of reported ophidian accidents, which usually occur in remote areas of health centers or hospitals, making patient care not timely". Authors conclude that "the four evaluated plant species could be used in the prevention of the symptoms of a bothrophic accident". However, and given that the authors propose "the consumption of the extracts sampled as a preventive measure against the effects of *Bothrops asper* venom in the absence of adequate and timely professional medical treatment", the i*n vitro* results should be supported by *in vivo* experimentation. Without these results, any conclusion is purely speculative and the recommendation of using these plant extracts can be more harmful than beneficial (by giving false confidence to the victim of a snake bite, thereby relegating more an appropriate but expensive medical treatment).

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Snake venomics and antivenomics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research